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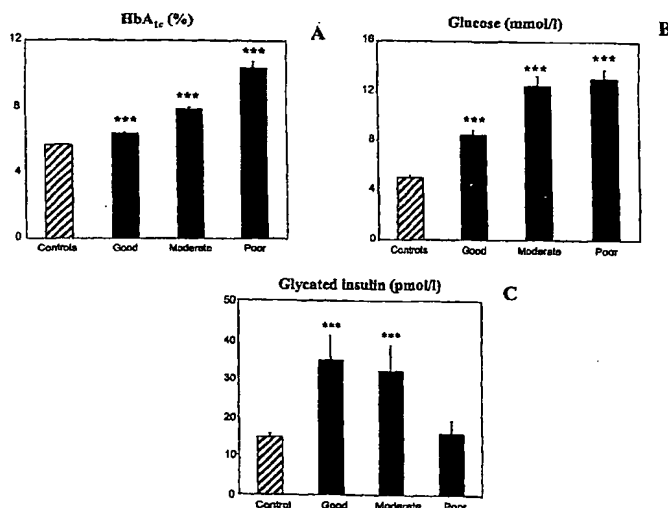
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(54) Title: **GLYCATED INSULIN AS A BIOMARKER FOR DIAGNOSIS AND MONITORING OF DIABETES**



(57) Abstract: One of the major pathophysiological consequences of long term elevation of plasma glucose in diabetes is an increase in the non-enzymatic glycation of proteins. Contrary to expectations the present inventors have determined that individuals with well controlled short duration diabetes have particularly high concentrations of glycated insulin which decrease with increased disease severity and duration of diabetes. Further, a small proportion of apparently normal healthy individuals exhibit high glycated insulin levels in line with expected incidence of diabetes in the population. Methods of predicting the onset of diabetes and for monitoring the progression of diabetes by measuring the concentration of Glycated Insulin and the progression of diabetes are disclosed.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

GLYCATED INSULIN AS A BIOMARKER FOR DIAGNOSIS AND MONITORING OF DIABETES

1 "Biomarker"

2

3 Field of the Invention

4

5 The present invention relates to the use of a novel
6 biomarker for the prediction and management of
7 diabetes mellitus together with its complications.
8 More particularly, the present invention provides
9 methods for predicting the onset of diabetes and for
10 monitoring disease progression.

11

12 One of the major pathophysiological consequences of
13 long term elevation of plasma glucose in diabetes is
14 an increase in the non-enzymatic glycation
15 (glycosylation) of proteins.

16

17 Formation of advanced glycation end-products (AGEs)
18 plays an important role in the long-term metabolic
19 consequences of diabetes, including ophthalmic,
20 renal and atherosclerotic vascular complications.
21 Glycation has been shown also to interfere with
22 normal cellular functions including the activities

1 of various enzymes such as Cu-Zn superoxide
2 dismutase.

3
4 Insulin is known to be glycated in the pancreatic
5 beta cells under conditions of hyperglycaemia. Thus
6 glycation results in an impairment of insulin
7 action.

8
9 Glycated insulin exhibits a reduced ability to
10 regulate plasma glucose homeostasis *in vivo* and to
11 stimulate adipose tissue lipogenesis and / or
12 glucose uptake and oxidation by isolated diaphragm
13 and abdominal muscle *in vitro*.

14
15 The site of glycation of human insulin has been
16 identified by electrospray tandem mass spectrometry
17 as the N-terminal Phe¹ of the B-chain.

18
19 Summary of the invention

20
21 It has long been accepted that, in diabetes, the
22 concentration of glycated proteins increases over
23 time, with disease progression and with increased
24 blood glucose concentration. For example, the
25 glycation of proteins such as haemoglobin has been
26 shown to increase as levels of blood glucose
27 increase in accordance with the severity and
28 duration of diabetes.

29
30 It has long been considered that, in a similar way,
31 the concentration of glycated insulin in the blood
32 increases with increasing blood glucose in diabetes.

1

2 Surprisingly, the present inventors have shown that,
3 contrary to expectations and the teaching of the
4 prior art, the concentration of glycated insulin
5 decreases with increased disease severity and / or
6 duration of diabetes.

7

8 Thus, according to a first aspect of the present
9 invention there is provided a method of monitoring
10 the progression of diabetes from a first timepoint
11 to a later timepoint, said method comprising the
12 steps:

13 providing a first biological sample obtained at
14 the first timepoint,

15 measuring the concentration of glycated insulin
16 in said biological sample,

17 providing a second biological sample obtained
18 at the later timepoint,

19 measuring the concentration of glycated insulin
20 in said second biological sample,

21 determining the difference in concentration of
22 glycated insulin between the first and second
23 biological samples, wherein a lower concentration at
24 the second timepoint is indicative of increased
25 disease severity and/or loss of control of blood
26 glucose.

27

28 Throughout this specification, references to glycated
29 insulin should be understood to refer to an insulin
30 molecule, a proinsulin molecule or fragments of
31 insulin or proinsulin linked to one or more
32 molecules of glucose, metabolites or related

1 reducing sugars in the form of glucitol adducts,
2 Schiff base adducts or amadori products.

3
4 Moreover, the demonstration that glycated insulin
5 concentrations are greater in early stage/well-
6 controlled diabetes than in later stage/poorly
7 controlled diabetes enables the use of glycated
8 insulin as an early indicator of diabetes. In
9 particular, the inventors' work suggests that
10 glycated insulin may be used in the early diagnosis
11 of diabetes e.g. prior to the appearance of other
12 symptoms and/or diagnostic markers such as
13 hyperglycaemia. Thus glycated insulin may be used in
14 diagnosis of diabetes prior to the appearance of
15 symptoms such as blood glucose levels in excess of
16 the normal range.

17
18 Prediabetes has been defined as the stage before the
19 development of diabetes, with normal glucose
20 tolerance but with an increased risk of developing
21 diabetes at some future time. Examples of increased
22 risk might include family history of diabetes, prior
23 diagnosis of gestational diabetes, presence of a
24 positive antibody test for diabetes, or insulin
25 resistance.

26
27 More recently, the American Diabetes Association has
28 suggested a new definition: "Pre-diabetes is the
29 state that occurs when a person's blood glucose
30 levels are higher than normal but not high enough
31 for a diagnosis of diabetes...Doctors sometimes
32 refer to this state of elevated blood glucose levels

1 as Impaired Glucose Tolerance or Impaired Fasting
2 Glucose (IGT/IFG), depending on which test is used
3 to detect it." Thus the criteria for prediabetes,
4 although not clearly defined, can be considered to
5 mean that an individual is on track to attain the
6 diagnostic criteria in the future.

7
8 Thus, in a second aspect, the present invention
9 provides a method of early diagnosis of diabetes or
10 a method of diagnosis of prediabetes in an
11 individual, the method comprising the steps:
12 providing a biological sample in which glucose
13 levels are within a normal range from said
14 individual,
15 measuring the concentration of glycated insulin in
16 the biological sample,
17 wherein the presence of glycated insulin at a
18 concentration greater than a predetermined minimum
19 is indicative of the presence of diabetes.

20
21 In the studies provided herein, as well as studying
22 the average levels of glycated insulin in control
23 subjects and diabetic subjects, the inventors also
24 examined the individual data for each subject.
25 Surprisingly, the inventors discovered that in the
26 control subjects, all of whom had been classified as
27 non-diabetic using conventional criteria (glucose
28 concentrations inside normal range).

29
30 This clearly suggests the invention may be further
31 used to predict the onset of diabetes and/or
32 identify individuals with a predisposition to

1 diabetes in advance of the appearance of any
2 symptoms conventionally used in the diagnosis of the
3 disease. Thus, the invention may be used in the
4 testing and monitoring of individuals believed to be
5 at risk of developing diabetes, e.g. individuals
6 with a family history of the disease in order to
7 enable early intervention to prevent onset or
8 development of the disease. Such testing and
9 monitoring may thus be used to identify or predict
10 the onset of diabetes weeks or months in advance of
11 the onset of the disease.

12
13 Thus, in a third aspect, there is provided a method
14 of predicting the onset of diabetes in an
15 individual, the method including the steps of:

16 providing a biological sample from said
17 individual,
18 measuring the concentration of glycated insulin in
19 the biological sample,

20 wherein the presence of glycated insulin at a
21 concentration greater than a predetermined minimum
22 is indicative of predisposition to diabetes.

23
24 As described above, the methods of the invention may
25 be used prior to the appearance of symptoms commonly
26 used in the diagnosis of diabetes. Thus, in
27 preferred embodiments of second and third aspects of
28 the invention, the biological sample has a glucose
29 concentration within a normal range i.e. a range not
30 normally considered diagnostic of diabetes, for
31 example less than 11.1 mmol/l (20mg/dl).

32

1 In preferred embodiments, the biological sample has
2 a glucose concentration for a random plasma sample
3 preferably less than 10 mmol/l, even more preferably
4 less than 9 mmol/l, most preferably less than 8
5 mmol/l. Alternatively, the biological sample has a
6 glucose concentration in a fasting plasma sample
7 less than 7.0 mmol/l (126 mg/dl).

8
9 In preferred embodiments of the invention the
10 predetermined minimum concentration of glycated
11 insulin is at least 20 pmol/l, more preferably at
12 least 23 pmol/l, even more preferably at least 25
13 pmol/l, most preferably at least 28 pmol/l.

14
15 Thus in preferred embodiments preferably glycated
16 insulin is considered to be raised if it is greater
17 than 20 pmol/l, preferably greater than 25 pmol/l,
18 more preferably greater than 28 pmol/l and even more
19 preferably greater than 30 pmol/l.

20
21 In preferred embodiments blood component
22 concentrations, e.g. glucose or glycated insulin are
23 measured in non fasted plasma.

24
25 In certain preferred embodiments of the second and
26 third aspects of the invention, the predetermined
27 minimum concentration is the concentration of
28 glycated insulin measured in a sample from the same
29 individual at an earlier timepoint. Thus these
30 aspects may be used in the monitoring of disease
31 progression and/or beta cell damage/dysfunction in an
32 individual.

1
2 Without being limited to any one theory, the early
3 elevation in glycated insulin levels may be
4 indicative of early stage dysfunction of or damage
5 to pancreatic beta cells, prior to significant
6 elevation of blood glucose. Thus, the invention
7 further provides a method of evaluating the
8 competency of the pancreatic beta cells of an
9 individual, the method comprising the step of
10 providing a biological sample, (e.g. blood, serum or
11 plasma) from the individual, and determining the
12 level of glycated insulin in the sample, wherein the
13 presence of glycated insulin in the sample at a
14 concentration in excess of a predetermined minimum
15 is indicative of dysfunction of or damage to the
16 beta cells.

17
18 Prediction of the onset of the disease permits early
19 counselling and intervention. Early detection of
20 the disease enables patient treatment and management
21 at an early stage.

22
23 In the invention, the presence and quantification of
24 glycated insulin in the sample may be carried out
25 using any assay known in the art. For example, such
26 assays may include, but are not limited to, ELISA
27 assays, or radioimmunoassay (RIA).

28
29 Other preferred methods of detecting the presence of
30 and quantifying glycated insulin includes IRMA
31 (immunoradiometric) assays and mass spectrometry

1 techniques. The procedures for carrying out such
2 assays are known in the art.

3

4 In a fourth aspect, the invention provides the use
5 of glycated insulin as a predictive marker for
6 glucose intolerance and/or diabetes.

7

8 In a fifth aspect, the invention provides the use of
9 glycated insulin as a predictive marker for
10 prediabetes or to predict the onset of diabetes.

11

12 Glucose intolerance is considered to be the stage
13 prior to overt diabetes in that a random test of
14 glucose concentration may not give a value high
15 enough to fall within the diabetes diagnostic
16 criterion. Prediabetes is a stage even before a
17 clear demonstration of glucose intolerance.

18

19 In a sixth aspect, there is provided an in vitro
20 assay method for detecting the presence of glycated
21 insulin in a biological sample, in which glucose
22 levels are normal, said assay method comprising the
23 steps:

24 providing a biological sample;
25 measuring the concentration of glycated insulin in
26 the biological sample;
27 wherein the presence of glycated insulin at a
28 concentration greater than a predetermined minimum
29 is indicative of diabetes or predisposition to
30 diabetes.

31

1 In a seventh aspect, there is provided an assay kit
2 for carrying out a method of the invention;
3 said kit comprising at least one antibody with
4 binding specificity to glycated insulin, means to
5 detect binding of the at least one antibody to
6 glycated insulin, details of a concentration range
7 of glycated insulin considered to be elevated from
8 normal levels , and instructions on how the assay is
9 to be performed and how the results are to be
10 interpreted.

11
12 Preferably, the instructions are such that the
13 finding of a decrease in glycated insulin levels
14 from a first sample obtained from a patient to a
15 later sample obtained from the patient is indicative
16 of increased disease severity and/or loss of glucose
17 control.

18
19 The invention provides the use of any of the above
20 methods to diagnose if a patient is glucose
21 intolerant.

22
23 In one embodiment glycated insulin is part of an
24 assay for a range of metabolites and substrates
25 relating to a number of diseases to determine the
26 disease status of individuals.

27
28 The invention further provides the use of glycated
29 insulin to monitor and record diabetic status.
30

1 Preferred features of each aspect of the present
2 invention are the same for the other aspects of the
3 invention, *mutatis mutandis*.

4

5 The invention will now be described, by way of
6 example only, with reference to the accompanying
7 figures, wherein:

8

9 Figure 1 shows glycated haemoglobin (HbA_{1c}),
10 plasma glucose and glycated insulin
11 concentrations of control subjects and diabetic
12 patients exhibiting good, moderate or poor
13 metabolic control. Characteristics and numbers
14 in each group are given in Table 1. Values are
15 mean \pm SEM. *** $p < 0.001$ compared with control
16 subjects.

17

18 Figure 2 shows individual glycated insulin
19 concentrations of 75 of the control non
20 diabetic subjects showing 4 individuals with
21 levels in excess of 2 standard deviations from
22 the overall group mean - other features of this
23 group are provided in Table 1 and figure 1.

24

25 **EXAMPLE 1**

26

27 Mid-morning blood samples were withdrawn from type 2
28 diabetic subjects (n=102) attending routine hospital
29 review appointments. All subjects were controlled by
30 diet alone or by oral hypoglycaemic agents. No
31 subjects were receiving insulin. Type 2 diabetic
32 subjects were divided into 3 groups depending upon

1 their glycaemic control. Group 1 represented
2 subjects under good glycaemic control with a HbA_{1c} <:
3 7% (upper limit of non-diabetic range, 6.5%). Group
4 2 was comprised of type 2 diabetic subjects with
5 moderate glycaemic control (HbA_{1c} 7-9 %) and Group 3
6 represented subjects with a HbA_{1c} > 9% (poor
7 glycaemic control). Age- and sex-matched normal
8 healthy individuals served as controls. This study
9 was approved by the ethics committee and carried out
10 following informed consent from all subjects.

11

12 A specific RIA for glycated insulin has been
13 developed. In brief, an N-terminally glycated
14 synthetic insulin peptide, closely related to the
15 amino-terminal sequence of the insulin B-chain (Phe-
16 Val-Asn-Gln-His-Leu-Tyr-Lys) was used to raise
17 specific antibodies in rabbits and guinea pigs. This
18 peptide comprised the naturally occurring 1-6
19 sequence of insulin B-chain with a Tyr and Lys
20 substituted at positions 7 and 8 respectively. For
21 determination of glycated insulin, the insulin
22 peptide was glycated under hyperglycaemic reducing
23 conditions and iodinated using the solid phase
24 iodogen method, generating a high specific activity
25 mono-iodinated I¹²⁵-tyrosylated glycated peptide
26 tracer. A glycated insulin antiserum was used to
27 establish a dextran-coated charcoal RIA with a
28 glycated human insulin standard curve in the
29 presence of insulin free serum. The glycated
30 insulin antibody cross-reacted 56% with glycated
31 proinsulin but cross-reaction with non-glycated

1 insulin, proinsulin and other pancreatic hormones
2 was negligible. Serum insulin was determined using a
3 routine radioimmunoassay with human insulin
4 standard. Glucose concentrations were determined
5 using the glucose oxidase method and HbA_{1c} was
6 measured in whole blood by ion-exchange HPLC. Serum
7 creatinine was determined using a multilayered dry
8 slide aminohydrolase technique.

9
10 Data are expressed as mean \pm SEM. Significant
11 differences between groups of data were assessed
12 using the unpaired Student's *t* test and statistical
13 significance was assumed if $p < 0.05$.

14
15 Figure 1 illustrates that glycated insulin
16 concentrations of diabetic patients exhibiting good
17 and moderate metabolic control are raised relative
18 to HbA_{1c}, plasma glucose and healthy control
19 subjects.

20
21 The characteristics of the 75 control subjects and
22 102 diabetic patients are summarised in Table 1.
23 Control and diabetic groups were well matched for
24 age and sex. Serum creatinine concentrations were
25 similar, indicating freedom from renal disease. BMI
26 of diabetic groups was increased compared with
27 controls. Duration of diabetes increased
28 progressively in good, moderate and poorly
29 controlled groups. A total of 70 diabetic subjects
30 were taking oral hypoglycaemic agents of whom 16
31 were taking metformin alone, 27 sulphonylurea alone
32 and 22 on a combination of metformin and

1 sulphonylurea. The remaining patients were treated
2 with dietary restriction alone (31%) or a
3 combination of other treatments (5%), which included
4 acarbose. The distribution of patients in each group
5 on these treatment regimes is given in Table 1.

6 Insulin concentrations measured in the control,
7 good, moderate and poorly controlled groups were 148 ± 19 , 191 ± 25 , 225 ± 40 and 159 ± 40 pmol/l,
8 respectively.
9

10 Control subjects had mean glycated haemoglobin
11 values of $5.7 \pm 0.1\%$ compared with 6.4 ± 0.1
12 ($p < 0.001$), 7.9 ± 0.1 ($p < 0.001$) and $10.4 \pm 0.4\%$
13 ($p < 0.001$) for good, moderate and poorly controlled
14 diabetic groups, respectively (Figure 1, graph A).
15 Plasma glucose concentrations were similarly raised
16 in the diabetic groups, with highest values observed
17 in the poorly controlled group (Figure 1, graph B).
18 A positive correlation between glycated haemoglobin
19 and glucose concentration was evident in the
20 combined groups ($r = 0.322$; $p < 0.01$).

21
22 As shown in Figure 1, graph C, plasma glycated
23 insulin concentration of good and moderately
24 controlled diabetic groups were increased 2.4-fold
25 ($p < 0.001$) and 2.2-fold ($p < 0.001$) compared with
26 control subjects. Plasma glycated insulin
27 concentrations in the control group, well,
28 moderately and poorly controlled diabetic groups
29 were 12.8 ± 1.1 (SD 9.2), 29.8 ± 5.4 (SD 35.7), 27.3
30 ± 5.7 (SD 36.5) and 13.5 ± 2.9 (SD 11.9) pmol/l,

1 respectively. Glycated insulin concentrations were
2 higher in the well controlled group with the lowest
3 glucose concentration and the shortest duration of
4 diabetes. This is surprising as increased glucose
5 levels are thought to promote glycation of proteins.
6 Thus, as observed for glycated haemoglobin, the
7 amount of glycated insulin would be expected to
8 increase in keeping with a time and concentration
9 dependent process in the pancreatic beta cell as
10 disclosed in the art.

11

12 The unexpected observation of a higher concentration
13 of glycated insulin in the test group with the
14 lowest glucose concentration lead the inventors to
15 conclude that glycated insulin is an early marker of
16 diabetes and its potential complications.

17

18 **Example 2**

19

20 The individual data for each subject was examined.
21 The results are shown in Figure 2. As can be seen,
22 surprisingly, the inventors discovered that in the
23 control subjects, all of whom had been classified as
24 non-diabetic using conventional criteria, (glucose
25 concentrations of control subjects were within
26 normal range of glucose concentration) there were
27 four out of the seventy-five subjects who
28 nevertheless displayed glycated insulin
29 concentration in excess of two standard deviations
30 of the overall mean. This interestingly correlates
31 with the incidence of the diabetes in the population
32 at large, which is between 3-5%. The inventors

1 conclude that as one would expect that 2-4
2 individuals in the population size used in the study
3 would go on to develop diabetes that this provides
4 evidence that increased glycated insulin
5 concentration is indicative of prediabetes.

6
7 The results clearly show that measurement of
8 glycated insulin in non-diabetic subjects, in which
9 glucose is not significantly raised, can be used to
10 predict the onset of diabetes and/or identify
11 individuals with a predisposition to diabetes in
12 advance of the appearance of any symptoms
13 conventionally used in the diagnosis of the disease.

14
15 Glycated insulin is suggested to be a feature of
16 beta cell dysfunction and insulin resistance in
17 diabetes.

18
19 Furthermore, glycated insulin is suggested to act as
20 an insulin receptor antagonist.

21
22 Unlike for example glycated haemoglobin levels, the
23 concentration of glycated insulin measured correlate
24 inversely with the degree of hyperglycaemia and most
25 importantly the duration of diabetes. This
26 surprising observation indicated that elevation of
27 glycated insulin/proinsulin is an early feature of
28 beta cell dysfunction and diabetes occurring before
29 increased glucose concentration and thus glycated
30 insulin can be used as a prediction method of
31 diabetes.

32

1 Glycated insulin concentration is dependent not only
2 on the glycaemic environment but also the ability of
3 the beta cells to transport and metabolise glucose
4 to reactive phosphorylated forms at the
5 intracellular sites of (pro)insulin synthesis and
6 storage.

7
8 Additionally, the secretory activity and competency
9 of the beta cells together with metabolic clearance
10 rate will also determine the rates of delivery and
11 removal of glycated insulin from the circulation.

12 It is notable that these various parameters are
13 deranged in prediabetes and in individuals with
14 increased risk of developing diabetes.

15 It is proposed that beta cell competency is a
16 determinant of the total plasma concentration of
17 glycated insulin in individual patients depending on
18 the status of their current disease.

19
20 A typical pattern of loss of beta cell competency,
21 followed by increased concentration of glycated
22 insulin, increased glucose concentration and loss of
23 first phase glucose-induced insulin secretion
24 followed by progressively impaired second-phase
25 insulin secretion, with complications for example
26 glucose blindness, and disproportionate
27 hyperproinsulinaemia with impaired basal or steady
28 state insulin secretion is proposed.

29

30 It is known that glucose concentration does not
31 correlate with the incidence of complications, for

1 example glucose blindness, which are well known in
2 the art. It is proposed that measurement of
3 glycated insulin provides an indicator of the
4 progression of diabetes, which can be used to
5 determine if complications are likely to arise.

6 Glycated insulin/proinsulin therefore provides a
7 novel and potentially important biomarker with
8 potential in the prediction, and management of
9 diabetes together with its complications.

10 Although the present invention has been particularly
11 shown and described with reference to a particular
12 example, it will be understood by those skilled in
13 the art that various changes in the form and details
14 may be made therein without departing from the scope
15 of the present invention.

1 **Table 1:** Characteristics of controls subjects and
 2 type 2 diabetic patients.

| | Control subjects | Patients with good control | Patients with moderate control | Patients with poor control |
|------------------------------|------------------|----------------------------|--------------------------------|----------------------------|
| Number of subjects | 75 | 44 | 41 | 17 |
| Male/female ratio | 31/44 | 21/23 | 24/17 | 9/8 |
| Age (years) | 59.5 ± 2.1 | 64.6 ± 1.4 | 63.4 ± 1.7 | 61.0 ± 3.7 |
| Duration of diabetes (years) | -- | 4.8 ± 0.6 | 7.1 ± 0.9 | 8.5 ± 1.3 |
| BMI (kg/m ²) | 25.6 ± 0.5 | 29.6 ± 1.0*** | 30.6 ± 1.0*** | 28.9 ± 1.3** |
| Serum Creatinine (μmol/l) | 86.0 ± 4.0 | 89.2 ± 3.6 | 89.0 ± 4.0 | 85.8 ± 3.6 |
| Diet (%) | --- | 40.9 | 26.8 | 17.6 |
| Metformin (%) | --- | 18.2 | 14.6 | 11.8 |
| Sulphonylureas (%) | --- | 29.5 | 26.8 | 17.6 |
| Metformin/Sulphonylureas (%) | --- | 6.8 | 26.8 | 47.1 |
| †Other drug combinations (%) | --- | 4.6 | 4.9 | 5.9 |

3
 4 Values are Mean ± SEM. ** $p < 0.01$, *** $p < 0.001$ compared
 5 with control subjects. †Other treatment combinations
 6 included metformin and acarbose, gliclazide and
 7 acarbose, and glibenclamide and acarbose.

1 **Claims**

- 2
- 3 1. A method of monitoring the progression of
- 4 diabetes from a first timepoint to a later
- 5 timepoint, said method comprising the steps
- 6 providing a first biological sample
- 7 obtained at the first timepoint,
- 8 measuring the concentration of glycated
- 9 insulin in said biological sample,
- 10 providing a second biological sample
- 11 obtained at the later timepoint,
- 12 measuring the concentration of glycated
- 13 insulin in said second biological sample,
- 14 determining the difference in
- 15 concentration of glycated insulin between
- 16 the first and second biological samples,
- 17 wherein a lower concentration at the
- 18 second timepoint is indicative of
- 19 increased disease severity and/or loss of
- 20 control of blood glucose.
- 21
- 22 2. A method of early diagnosis of diabetes in an
- 23 individual, the method comprising the steps
- 24 providing a biological sample in which glucose
- 25 levels are within a normal range from said
- 26 individual,
- 27 measuring the concentration of glycated insulin
- 28 in the biological sample,
- 29 wherein the presence of glycated insulin at a
- 30 concentration greater than a predetermined minimum
- 31 is indicative of the presence of diabetes.
- 32

- 1 3. A method of predicting the onset of diabetes in
2 an individual, the method including the steps
3 of;
4 providing a biological sample from said
5 individual,
6 measuring the concentration of glycated insulin
7 in the biological sample,
8 wherein the presence of glycated insulin at a
9 concentration greater than a predetermined
10 minimum is indicative of predisposition to
11 diabetes.
12
- 13 4. The method according to claim 3, wherein the
14 concentration of glucose in the biological
15 sample is within the normal range.
16
- 17 5. The method according to claim 2 or claim 4
18 wherein the normal range of glucose is less than
19 11.1 mmol/l in a random plasma sample.
20
- 21 6. The method according to any one of claims 2 to 5
22 wherein said predetermined minimum concentration
23 is the concentration of glycated insulin
24 measured in a sample from the same individual at
25 an earlier timepoint.
26
- 27 7. The method according to any one of claims 2 to
28 6, wherein said predetermined minimum
29 concentration of glycated insulin in a non
30 fasted sample is at least 20 pmol/l.
31

- 1 8. A method as claimed in any preceding claim
2 wherein glycated insulin in the sample is
3 measured by means of radioimmunoassay (RIA).
4
- 5 9. Use of glycated insulin, as a predictive marker
6 for glucose intolerance and/or diabetes.
7
- 8 10. Use of glycated insulin as a predictive marker
9 for prediabetes or to predict the onset of
10 diabetes.
11
- 12 11. An *in vitro* assay method for detecting the
13 presence of glycated insulin in a biological
14 sample, in which glucose levels are normal, said
15 assay method comprising the steps:
16 providing a biological sample;
17 measuring the concentration of glycated insulin
18 in the biological sample;
19 wherein the presence of glycated insulin at a
20 concentration greater than a predetermined
21 minimum is indicative of diabetes or
22 predisposition to diabetes.
23
- 24 12. The method according to claim 11, wherein the
25 predetermined minimum is the concentration of
26 glycated insulin measured in a sample from the
27 same individual at an earlier timepoint.
28
- 29 13. The method according to claim 11 or claim 12
30 wherein the predetermined minimum concentration
31 in a non fasted sample is at least 20 pmol/l.
32

1 14. An assay kit for carrying out a method according
2 to any one of claims 1 to 8 or 11 to 13;
3 said kit comprising at least one antibody with
4 binding specificity to glycated insulin, means
5 to detect binding of the at least one antibody
6 to Glycated insulin, details of a concentration
7 range of Glycated insulin considered to be
8 elevated from normal levels, and instructions on
9 how the assay is to be performed and how the
10 results are to be interpreted..

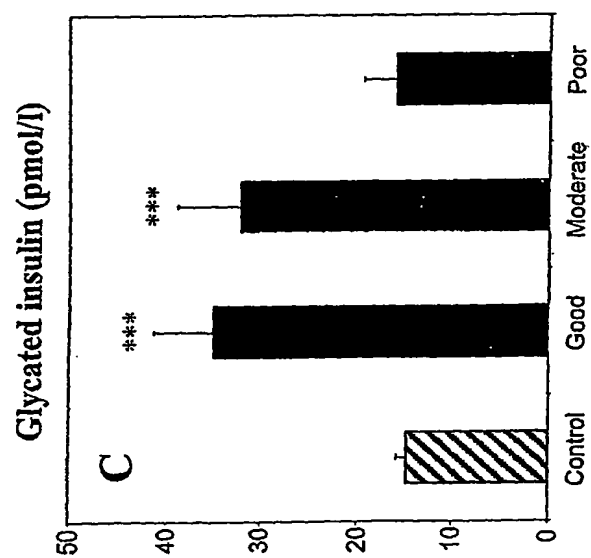
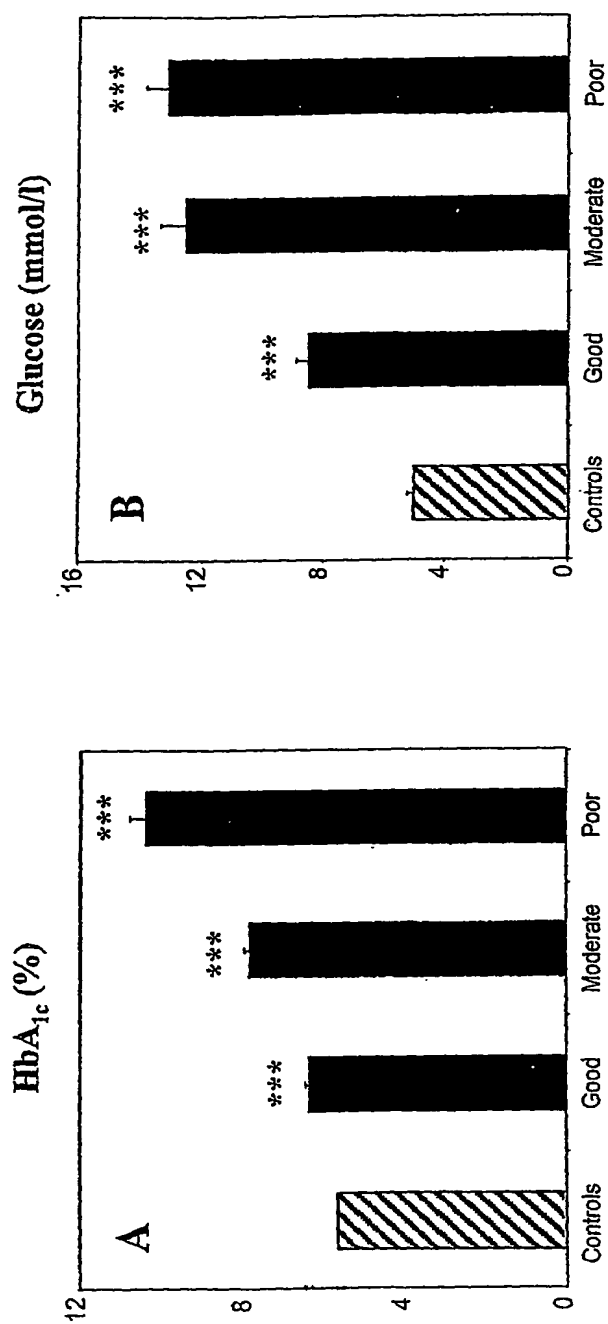
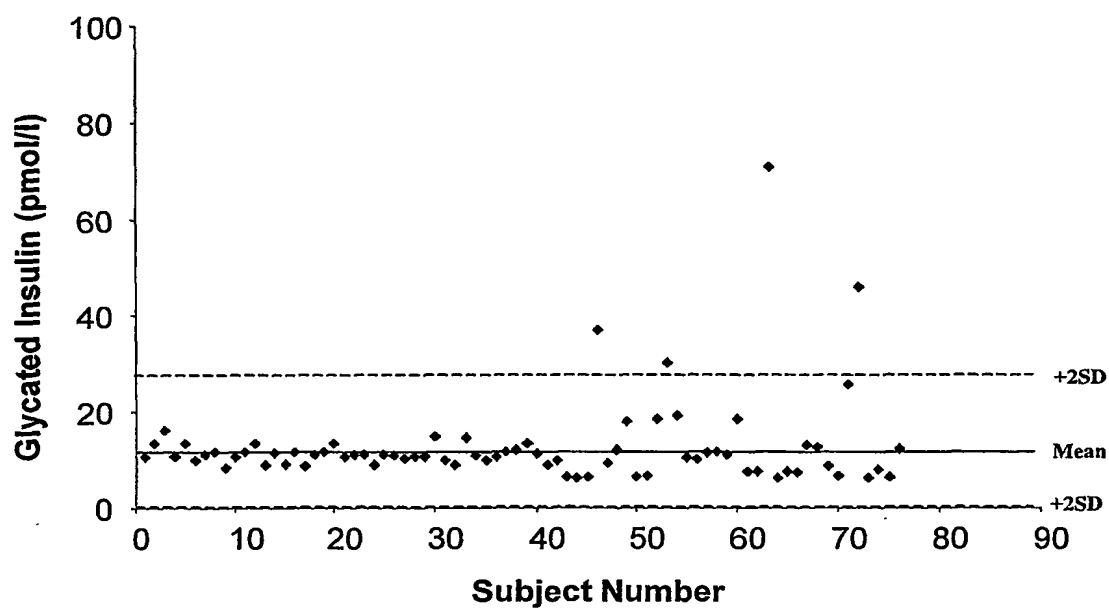
**Figure 1**

Figure 2: Circulating glycated insulin in healthy control subjects



INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/04128

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/66 G01N33/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search

15 January 2004

Date of mailing of the international search report

13/02/2004

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INTERNATIONAL SEARCH REPORT

Int. Patent Application No

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